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Depression of the Neural Taste Responses by Chloroform and Bromoform in Fish

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To check effectiveness of chloroform and bromoform on the taste receptive membranes of carp (*Cyprinus carpio* L.), we recorded electrophysiologically the neural taste responses to L-amino acids during applications of several concentrations of the two chemicals in the artificial fresh water bathing the taste epithelia. The two compounds slightly elicited neural excitation by themselves, and have some effects on taste responses. Taste responsiveness for L-Ala and L-MSG were significantly depressed with the increase of concentration of chloroform and bromoform (0.001~0.1 mg/l). It is obvious that the latter affected the taste responses more strongly than the former does at the same concentrations. These results indicate that although chloroform and bromoform depressed the neural responses of the taste system somehow, it is not clear from the present experiments that these chemicals might cause anesthetic state on the taste membrane.

Key words : taste, depression, trihalomethanes, electrophysiology, fish

Introduction

Chloroform and bromoform, halogenated ethers, have been known to be anesthetics and also to be trihalomethanes (THMs) in drinking water. The latter was known as byproducts of the disinfection process for tap water (TW) chlorination. The two halogenated compounds have been investigated to affect on the central nervous system, and interact in some sort of attractive (binding) way with proteins[1]. These have been indicated to have important functional interaction with N-methyl-D-aspartate (NMDA) class of glutamate receptor[2,3], and depressed glutamate stimulation of MK-801 (channel blocker for NMDA receptor) binding in a concentration-dependent manner[2]. This inhibition of MK-801 binding site is supposed not to be caused by competition with respect to glutamate[2,3]. It was also reported in mouse cortical wedges that general anesthetics including chloroform antagonized both AMPA (alpha-amino-3-hydroxy-5-methyl-4-isoxazol propionate) and NMDA responses, and the antagonism of the excitatory amino acids responses exerted by those drugs was non-competitive in nature[4]. However, volatile anesthetics including halogenated ethers were noted to effect all cells and their mechanisms and sites of action remain unknown[2,5]. On the other hand, chlorination for the water supplies has almost completely eliminated from our lives the risks of waterborne diseases, but possible risks from THMs are also indicated as one of toxics[6,7],

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which detrimentally affect the liver, kidney and central nervous system and also causes cancers [7~14]. However, it is assumed that THMs do not pose a high health risk compared to those waterborne diseases. Since water quality standards of drinking water for THMs were publicly imposed, such compounds may be present in virtually all chlorinated water supplies[12,14].

We hypothesized here that such chloroform and bromoform may cause some effect on epithelial membrane of aqueous animals, especially sensory ones. To clarify this hypothesis, we decided to use the taste system of fish as a bioassay system, because it is easily conceivable that such chemicals must have some effects on biological membranes. To check their effectiveness on the taste system of experimental animals, we recorded electrophysiologically the neural taste responses to L-amino acids, important cues for fishes[16~19], during applications of several concentrations of chloroform and bromoform in the artificial fresh water (AFW) bathing the taste epithelia.

Materials and Methods

Twelve carp (*Cyprinus carpio* L.), approximately 20-25 cm in body length, were paralyzed with an intramuscular injection of Flaxidil (gallamine triethiodide, 0.5 mg/kg body weight) and positioned on a wax plate in a Plexiglas container. Aerated distilled water containing the anesthetic, 0.02% MS-222 (ethyl-*m*-aminobenzoate methane sulfonic acid) continuously perfuse the gills throughout the experiments. Supplemental Flaxedil was administrated as required. The electrophysiological set-up was the same as described previously[16]. Briefly, a branch of the trigemino-facial complex nerve that innervates the maxillary barbel was severed within the eye socket and its perpheral cut end was placed on an Ag-AgCl wire electrode. The electrical activities of the whole nerve bundle were amplified, monitored aurally, integrated (0.5 sec) and displayed on an oscilloscope. Data acquisition occurred via PowerLab/8s (AD Instrument Pty Ltd.). The digitized data files were analyzed off-line with computer software (Chart 4, AD Instrument Pty Ltd.).

To avoid possible deleterious effects from the TW containing in virtually THMs, AFW, (3.0 NaCl, 0.2 KCl, 0.2 $CaCl_2$, and 0.2 $MgCl_2$ in mM ; adjusted to pH 7.0 with 1mM HEPES) modified from Michel's[20] was used to bathe the taste receptive fields, barbels and lips. Stock solutions, L-Ala and mono-sodium L-Glu (L-MSG) were prepared weekly at 10 mM in DW, which were stored at 4 ℃. The desired experimental concentrations were prepared daily by dilution with AFW. Solutions of chloroform and bromoform were also prepared with AFW. The stimulating apparatus and methodology were the same as previously described[16]. Most test comparison of the relative stimulatory effectiveness of amino acids occurred at 1 mM with at least 5 min inter-stimulus intervals. The standard, 1 mM L-Ala was applied regularly to check the reproducibility of the preparations. The magnitudes of the taste responses were measured as the peak heights of the integrated responses. A paired sample *t*-test (two-tailed) was used to test the significance of the paired means.

Results

The effectiveness of chloroform and bromoform on neural taste responses of carp to L-Ala and L-MSG was examined with electrophysiological techniques in this study. The concentrations of the two halogenated chemicals used to continuously bather the taste receptive fields in general were assigned based on water utility measurements of several nations. The values regulated by the new water quality standards of Japan in 2004 for chloroform and

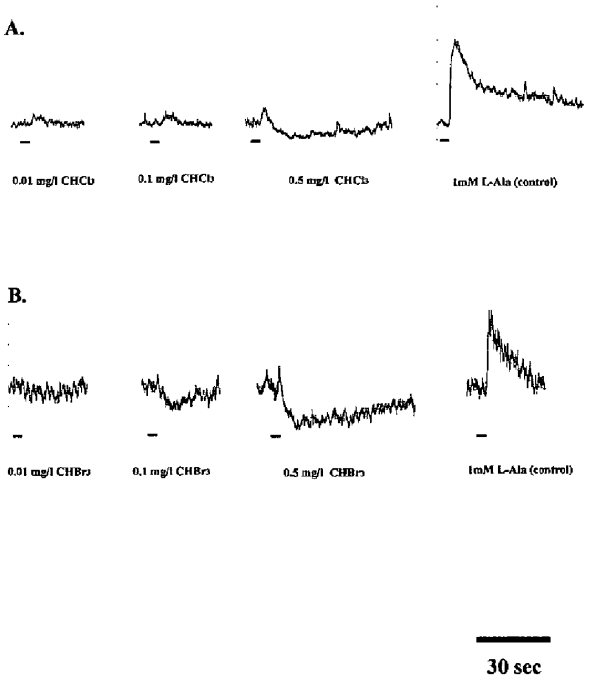

Fig. 1
Typical neural taste recordings of integrated responses to several concentrations of chloroform (A) and bromoform (B). Integrated responses to 1 mM L-Ala are shown as a control in the right edges of A and B.

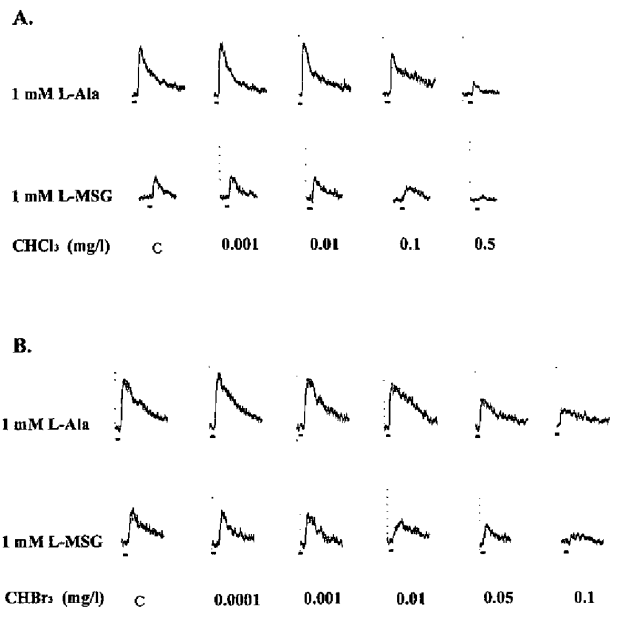

Fig. 2
A : Effects of chloroform on taste responses.
B : Effects of bromoform on taste responses.
Abbreviation: c, control responses to 1 mM L-Ala without $CHCl_3$ or $CHBr_3$.

bromoform are 0.06 and 0.09 mg/l, respectively [12]. The present determination of the effects of chloroform and bromoform on taste responses in carp was performed over the range of several concentrations that included these standards. Chloroform and bromoform elicited neural excitation of trigemino-facial complex nerve twigs (Fig. 1) by themselves. They elicited some slight responses at the concentration of 0.01 mg/l, but the baselines of the recordings were apparently shifted to the positive over at 0.5 mg/l of chloroform or 0.1 mg/l of bromoform (Fig. 1 A & B). The two chemicals have also some effects on taste responses (Fig. 2), where depression of taste responses to L-Ala and mono-sodium L-Glu (L-MSG) was observed as shown in Fig. 2. Decrementation of the responses for two amino acids started in the presence of 0.1 mg/l of chloroform and 0.01 mg/l of bromoform, respectively (Fig. 2). Taste response magnitudes for the two amino acids were significantly decreased with the increase of concentrations of chloroform and bromoform, above 0.001~0.01 mg/l ($p<0.05$), where significant difference of taste responses from the standard (STD in Fig. 3) was only observed for responses to L-MSG at the lowest of 0.001 mg/l of bromoform (Fig. 3) ($p<0.05$). It is obvious that bromoform affected strongly to the taste responses than chloroform does at the same concentrations (Fig. 2 & 3). However, these depressions of relative response magnitudes of taste with the two chemicals were reversible.

The contaminant being supposed to be THMs in the TW is causing some effect, which elicited some neural responses for the nerve recordings (Fig. 1 A & B). As the responses were decreased in the presence of chloroform and bromoform (Fig. 2 & 3) and TW compete with the two chemicals on the neural activity elicited (Fig. 4 A), the contaminant might be possible to be one of

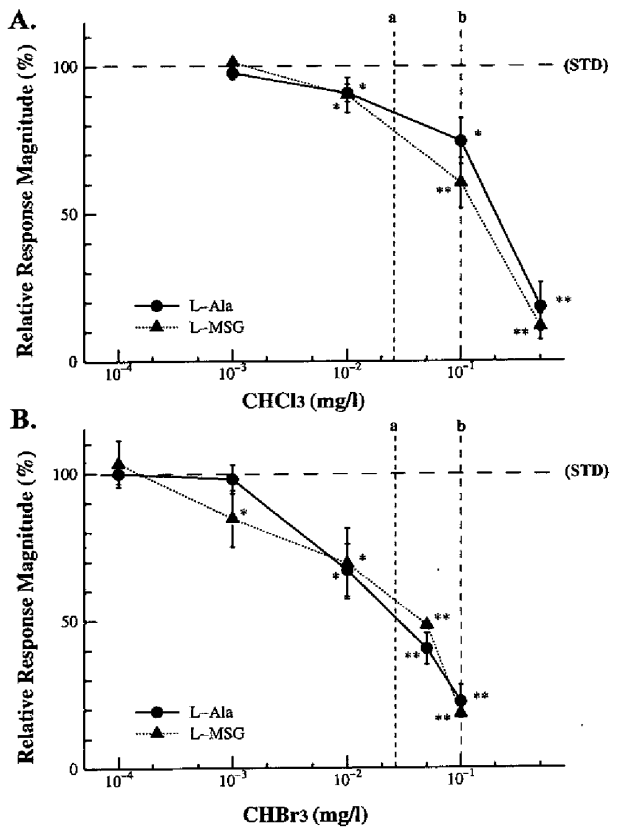

Fig. 3

Effects of chloroform (A) and bromoform (B) with increase of their concentrations to taste responses. Significant differences between standards (STD, relative response magnitude for 1 mM L-Ala or L-MSG) and the responses depressed were tested by a paired sample *t*-test (two-tailed).

Abbreviations : *, significant ($p<0.05$) ; **, significant ($p<0.01$) : NS, not significant ; STD, standard ; a, The German standard of total THMs for drinking water ; b, Japanese Standard of total THMs for drinking water.

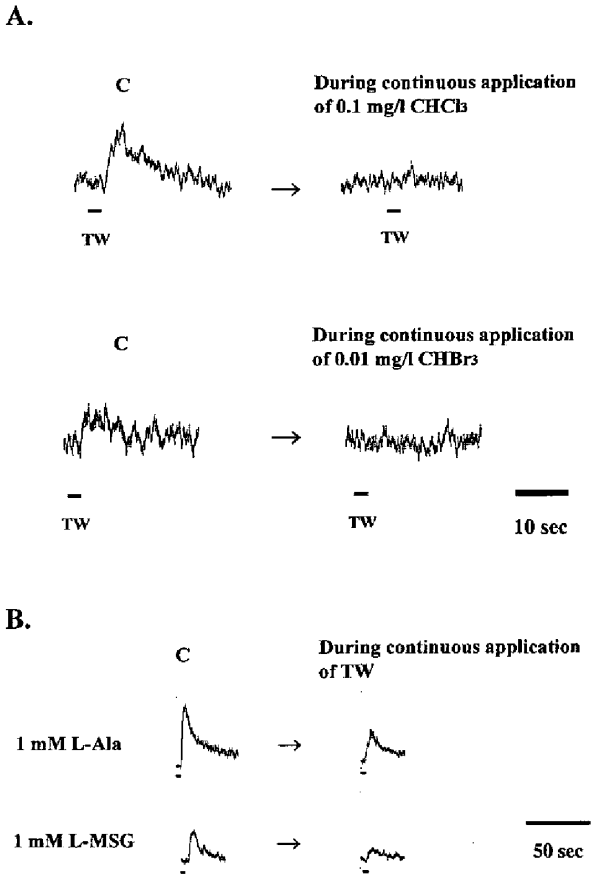

Fig. 4

A : Competition experiment of TW vs. $CHCl_3$ or $CHBr_3$. $CHCl_3$ and $CHBr_3$ depressed TW responses.

B : Depression of taste responses to 1 mM L-Ala and L-MSG with continuous application of TW.

Abbreviation : TW, tap water.

them or these two. The taste responses for 1 mM L-Ala and L-MSG (Fig. 4 B) were significantly decreased with the current flow of TW ($p<0.01$); i.e. the ratio of the decrementation of responses for the two amino acids by TW are 49.6 ± 7.0 and 33.5 ± 5.1 % (mean ± S.E., n=6) for 1 mM L-Ala and L-MSG, respectively. Each depression ratio from the control (without TW) was significantly different ($p<0.01$). The depression ratios between two chemicals are also significantly different ($p<0.05$), where the responses for L-MSG were apparently depressed than those for L-Ala with TW.

Discussion

This study indicates that chloroform and bromoform definitely affect the neural responses of the taste system at certain concentrations (Fig. 2 & 3). Although the baselines of the recordings were apparently shifted to the positive with bathing by certain concentrations of chloroform or bromoform (Fig. 1), the reason is not conceived by the present data. The diversity of the structures of simple anesthetic molecules (chloroform, bromoform, diethyl ether, or nitrous oxide) indicates that there are no com-

mon receptors, which is difficult to reconcile with the specific interaction concept[2,6]. It was also noted that inhaled anesthetic binding interaction are really weak and such low-affinity and high-capacity binding with those implies non-specific[1]. However, these anesthetics were suggested to interact with neural membrane proteins to alter their activity[1]. The interaction of anesthetics with the lipophilic (hydrophobic) domains was hypothesized to lead to decreased flexibility of proteins[2], which implicated the importance of marginal stability and conformational dynamics to protein function[1]. In addition, G-protein coupled receptors like amino acids' taste ones were suggested as direct targets of inhaled anesthetics[5], as well. A possibility of reversible inhibition to enhancing effect of enflurane, toluene, and chloroform on glycine and $GABA_A$ receptors (inhibitory neurotransmitter systems) illustrate the feasibility of antagonism of the effects of those chemicals[22~24]. It is conceivable that such enhanced activity of inhibitory and depression of excitatory (NMDA) neurotransmitter systems probably contribute to the anesthetic state[2,3]. This anesthetic state might occur on the taste receptive membrane as chloroform and bromoform affect on the taste membranes somehow. Thus, to analyze this state, mechanisms of the taste receptors should be clarified to reveal how chloroform or bromoform work on the surface membrane. On the other hand, it was shown that molecular volume is a key determinant of anesthetic activity, and pharmacological profile of bromoform was similar to that seen with halothane, but distinct from that observed for isoflurane and chloroform[25]. In this study, the depression ratios of taste responses by chloroform and bromoform were concentration-dependent. If this difference of the profiles between chloroform and bromoform is true even in this taste system, further experimental strategy is needed to clarify the difference in the future.

Byproducts in drinking water, such as THMs, are widely known to introduce some possible biological hazards to animals[9,15]. However, most toxicological studies have focused on a carcinogenic or reproductive toxic potential of THMs[9,13]. In addition, it has been thought that a relatively low association between animal exposure to total THMs in drinking water and the hazard is minimized, because the risk is unlikely to occur via drinking water[8,12,14]. Therefore, few physiological reports concerning toxicity of THMs in drinking water and animal hazards has been shown, especially in terms of sensory systems. Thus, the adverse effect of chloroform and bromoform as the representatives of THMs to taste responsiveness of carp to amino acids was elucidated here with neural taste recordings. Competition experiments between TW and 0.1 mg/l of chloroform or 0.01 mg of bromoform in this study (Fig. 3) suggest that at least they might be contained at concentrations effecting the taste membrane in the TW.

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